Study finds protein 'cement' that stabilizes the crossroad of chromosomes

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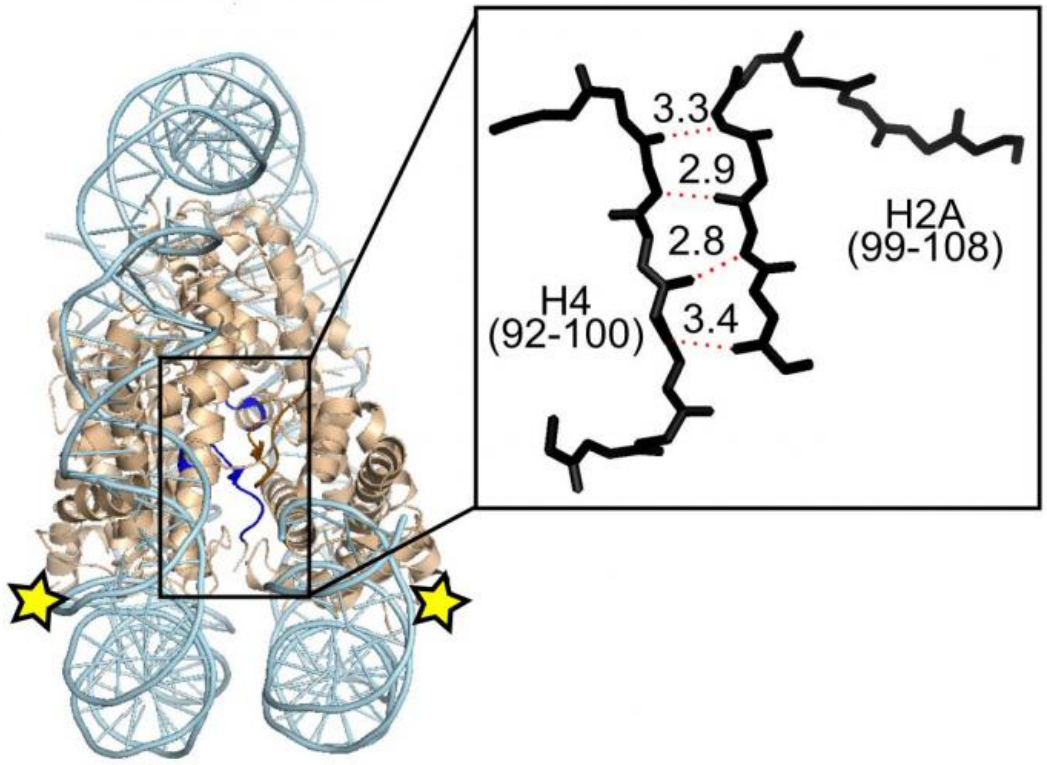

The direct binding of CENP-C to the CENP-A nucleosome stabilizes hydrogen bonding within internal secondary structures of the CENP-A nucleosome. Stars indicate fluorescent probes that move closer to each other upon CENP-C binding, as a result of CENP-C altering the shape of the CENP-A nucleosome into a more rigid and stable conformation. Credit: Lucie Guo and Ben Black, Perelman School of Medicine, University of Pennsylvania

Cell division is the basis of life and requires that each daughter cell receive the proper complement of chromosomes. In most organisms, this process is mediated at the familiar constricted intersection of X-shaped chromosomes. This area, called the centromere, is where special proteins gather and attach to pull daughter cells apart during cell division. The structure and biology of the centromere is of considerable scientific interest because problems with it can lead to abnormalities in the chromosomes of daughter cells, which are the basis of such disorders as Down syndrome.

A new study by researchers at the Perelman School of Medicine at the University of Pennsylvania published in *Science* this week describes how the centromere is stabilized during replication. DNA in the nucleus is packaged into protein/DNA complexes called nucleosomes. As it turns out, the centromere is distinguished not only by its DNA sequence but also by a special type of nucleosome, which includes a protein called CENP-A.

Senior author Ben E. Black PhD, associate professor of Biochemistry and Biophysics, and his Penn team described the structure of CENP-A almost five years ago. The question the investigators asked now was, how does the cell ensure that CENP-A-containing nucleosomes remain at, and thus continue to mark, the centromere during the massive changes a cell undergoes when it divides?

Simply put, it involves an accessory protein called CENP-C. "Overall, my lab is interested in better understanding the molecular basis of inheritance and the role of the centromere, as a 'control locus,' for maintaining heredity," says Black.

His team applied a battery of biophysical techniques to study the

structure and stability of CENP-A-containing nucleosomes in a test tube. Their data indicate that CENP-A-bearing nucleosomes have an unexpectedly flexible structure, adopting a relaxed conformation in the absence of CENP-C, and a more compact shape in its presence. This CENP-C-induced shape shift correlates with changes in how DNA wraps around the centromere's nucleosomes, making the structure similar to that found in living cells.

Their findings also address the question of the stability of CENP-A molecules at centromeres. Under normal conditions CENP-A binds centromeres and effectively never lets go. Indeed, when the authors tracked where proteins "reside" in live cells, they found that, unlike traditional nucleosomes that package the DNA throughout the rest of the chromosome, CENP-A-containing nucleosomes apparently never dissociate after newly generated CENP-A protein is first delivered to the centromere during a short time window following cell division. "The CENP-A is basically cemented at the centromere of origin," Black explains. But in cells lacking CENP-C, CENP-A dissociates readily, suggesting that CENP-C binding to CENP-A is what imparts that stability.

Investigators have known for the past 20 years that part of chromosome inheritance is controlled by epigenetics, implicating the protein spools around which DNA is wound as the driving force, rather than what is encoded in the DNA sequence itself. Those spools are built of histone proteins, and chemical changes to these spool proteins can either loosen or tighten their interaction with DNA. This, in turn, alters a gene's expression up or down. In the case of the centromere, it marks the site where spindle fibers attach independently of the underlying DNA sequence.

Earlier, Black and other chromosome researchers established that CENP-A is the key epigenetic protein at the centromere and replaces the

regular histone protein H3. CENP-A attracts other proteins, and in cell division builds a massive structure called the kinetochore, for pulling duplicated chromosomes apart during cell division.

Black notes that these data suggest a model of epigenetic biology distinct from the traditional view of nucleosomes as static scaffolds on which key functional molecules assemble. Instead, the team's data suggest that histone variants and post-translational modifications, which change the biological properties of nucleosomes through changes in shape (by adding or removing enzyme docking sites) make nucleosomes active participants in cell division and gene expression.

"This is conceptually very similar to thinking about how enzymes can be regulated—their activity can be turned on and off," he explains. "In this case, we're not talking about how enzymes affect a chemical reaction; we're talking about how the nucleosome and this entire part of the chromosome is stabilized. If stability is lost, then the chromosome and all the genes carried on it would not be delivered faithfully to each cell upon division. This is the sort of genetic catastrophe that is a hallmark of cancer cells. Or if it happens in the sperm or egg cell lines, it leads to spontaneous abortions or children with disorders such as Down syndrome."

This mode of nucleosome regulation and stabilization may well be common to other epigenetic processes, Black adds. Indeed, he says the results suggest that other histone variants and histone post-translational modifications may serve a similar function as the example at the centromere with CENP-A and CENP-C, for instance in the regulation of gene expression.

"I don't know how widely this occurs," he says, "but I'd be very surprised if this was the only place in nature that had evolved to take advantage of the fact that the shape of nucleosomes can be regulated by protein-

binding events."

Black says CENP-A-mediated stability could explain how oocytes retain the epigenetic information that preserves the fidelity of chromosome inheritance over so many years of fertility, and he is preparing to test that hypothesis now.

More information: "CENP-C reshapes and stabilizes CENP-A nucleosomes at the centromere", www.sciencemag.org/lookup/doi/ … 1126/science.1259308

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